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BULLETIN No. 64.

THE TOXICITY OF LIME TO FOMES LIGNOSUS KLOTZSCH.

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DEPARTMENT OF AGRICULTURE, CEYLON.

BULLETIN No. 64.

THE TOXICITY OF LIME TO FOMES LIGNOSUS KLOTZSCH.

DISEASE control in agricultural crops is one of the important cultural operations to which due attention must be paid to obtain the maximum harvest. In annual crops the loss of individual plants through disease reduces the crop for that year, a fresh sowing of seed providing the plants for the succeeding year. In perennial crops, however, the annual harvest is gathered from the same plants, and the crop returns thus depend on the continued healthy growth of each individual plant from year to year. The loss of individual plants in perennial crops is evidently a much more serious matter than in annual crops. Where the individual plants are trees, as in the case of the rubber tree, continued loss through the spread of disease from a focus of infection results in serious diminution of the stand of trees per acre, and thus in permanent reduction of the harvest.

The treatment of disease in temperate perennial crops has been the subject of much investigation. The prevalent diseases are generally of the stem, leaves, and fruit, and accordingly investigation has been directed principally to the preparation and application of fungicides, such as copper and lime sulphur sprays, for the above-ground portion of the plant. Root diseases are less common, and their treatment and control have received correspondingly less attention. Root diseases are, however, of frequent occurrence in the tropics, and are no less important and troublesome than stem diseases. The eradication of root disease, in fact, presents more difficulties than the eradication of many stem diseases. Preventive measures to stop the spread of root disease from diseased to healthy trees are particularly important, as it is usually impossible to save trees which have been attacked.

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Growth of the Fungus under Field Conditions.

Of the root diseases of rubber (*Hevea brasiliensis* Muell.—Arg.), the most widely spread and probably the most dangerous is that caused by the fungus *Fomes lignosus* Klotzsch. Petch* records that it occurs in Ceylon, South India, Java, Sumatra, Malaya, Borneo, West Africa, and the Congo region, and states that it has caused considerable loss, and involved large expenditure on its eradication. The fungus is indigenous in Ceylon, and is disseminated by wind-blown spores. These, given a suitable substratum, *e.g.*, old jungle stumps or other dead wood, germinate, and in time the mycelium of the fungus permeates the whole of the woody substratum at its disposal, and from this as a centre sends out fungus strands, which spread freely through the soil. Where these free-growing strands come in contact with healthy roots of adjacent trees, they penetrate them, and in due course the whole root system is attacked. Healthy roots in contact with the centre of infection are, of course, attacked even more quickly. Owing to the occurrence of these free-growing strands the fungus spreads rapidly, and many trees may be infected, before the disease is detected by the death of the tree first attacked. The fungus tends to spread down water-courses and ravines.

Control of the Fungus under Field Conditions.

It has been found by experience that it is extremely difficult to eradicate the fungus from large infected areas, even where the nature of the soil offers no obstacle to digging and trenching. If the disease occurs, as it frequently does, on rocky hill slopes planted up with rubber, it is practically impossible to arrest the spread of the fungus. The control measures usually adopted may be summed up as—

(1) Complete removal and destruction by fire of the root system of diseased trees.
(2) Isolation of the area of infection by a trench about 2 feet deep, which is kept clear of vegetation.
(3) Application of quicklime to the infected soil.

The efficacy of (1) depends on the extent to which roots are followed up and removed from the soil. Reasonable care and supervision should suffice to ensure a high degree of efficiency. It should be emphasized that this operation is equivalent to the removal of the source of infection, and it is thus the most important of these measures. The isolation

* Petch, T. Diseases and Pests of the Rubber Tree. 1921.

trench (2) is next in order of importance, and serves to stop the spread of free-growing fungus strands from infected material inadvertently overlooked. It further separates the bulk of the surface roots of healthy trees from contact with the infected area. As indicated above, however, neither of measures (1) and (2) can be applied, where the disease occurs on rocky soil, or can at most be only partially applied.

The application of quicklime to infected soil as a fungicide is fairly general. On rubber estates it is forked into the soil or scattered over the surface at the rate of about 60 lb. per tree, after the removal of the diseased trees. In some cases, after such applications of quicklime, the mycelium of the fungus has been found to persist in the soil apparently unharmed. In one instance a fructification of the fungus was found growing on a lump of lime, which had been applied to an infected area; this piece of lime, however, may have been re-converted to carbonate before the fructification appeared on it. The effect of quicklime on the fungus may be two-fold: (1) A scorching effect caused by direct contact; and (2) a toxic effect produced by the slaked lime in solution or by the alkalinity induced in the soil. As quicklime had been occasionally found ineffective in action in the field, it became desirable to investigate the effect of quicklime on the growth of the fungus, and to obtain information as to the growth of the fungus under acid and alkaline conditions. To this end the cultural experiments described hereafter have been carried out.

Isolation of the Fungus.

A young fructification of the fungus was obtained, from which a section was cut out. This was immersed in alcohol and flamed. The external parts were then cut away with a sterile knife, and small portions of the interior were transferred to Petri dishes containing bean agar. Pure cultures could be readily obtained in this way, the inoculum throwing out hyphæ in the course of two or three days. Sub-cultures were made from these isolation cultures, by transferring small blocks of the medium with the hyphæ attached to dishes containing neutralized culture medium, and from these further subcultures were made as required. The fungus was thus kept in artificial culture throughout the experiment. A neutralized medium was employed in the subcultures to avoid transferring small portions of medium of unknown reaction to acid and alkaline dishes when making the inoculations.

Preparation and Standardization of Culture Media.

Various nutrient agars were tried, including French bean, potato, maize, prune, and varieties of bean seeds (as distinct from French beans where young, fresh pods were used). The fungus grew best on French bean agar, forming a thick felted mycelium interspersed with strands; on maize and potato the growth was somewhat thinner. The plant decoctions were made up in accordance with Duggar's* formulæ, 20 grams of agar were added to each litre, and the solution was then filtered and standardized. The titrations were carried out with 25 cc. portions of the medium, decinormal sodium hydroxide being run in from the burette with phenolphthalein as indicator. The bulk of the medium was then neutralized by the addition of the requisite quantity of normal sodium hydroxide.

Some nutrient media, as, for example, French bean, have a marked brownish colour, which renders it difficult to detect colour changes in the indicator during titration. Colourless media, such as maize, which could be more accurately titrated, thus provided a useful check on growth measurements on bean media. Titrations were carried on till a decided pink colouration was obtained, and, as far as could be estimated, till the same intensity of pink was produced in each case, where direct inter-comparisons were to be made.

Method.

Growth measurements can be conveniently made on Petri dish cultures, which further permit of ready comparison between dishes in a series. The method at first adopted in preparing a series was to measure out by pipette 10 cc. portions of the neutral medium which were transferred to small flasks. The flasks were next autoclaved for 30 minutes at 115° C. The desired quantity of normal acid or alkali was then run in to each flask from the burette, and the contents of all were brought to a standard volume of 12 cc. by the addition of the requisite quantity of distilled water, for example, to the 0·1 cc. flask 1·9 cc. of water was added, to the 0·2 cc. flask 1·8 cc. water, and so on, while to the control flask 2 cc. of water were added. The Petri dishes were then poured and inoculated. The flask contents were made up to a standard volume, in order to eliminate the possible effect on growth of the varying dilution of the culture medium produced by the addition of varying quantities of acid or alkali. The control dishes were, therefore, made up to standard volume by the addition of 2 cc. of distilled water.

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* Duggar, B. M. Fungous diseases of Plants. 1909.

In order to eliminate contaminations that frequently occurred, the above procedure was modified, and the acid or alkali with the distilled water were added before autoclaving. It was found, however, that after autoclaving with all proportions of acid, the agar had lost the property of solidifying on cooling. In the final series, therefore, the flasks with the medium and the requisite quantity of water were autoclaved, the acid and alkali added afterwards, and the dishes poured immediately. This resulted in occasional contamination, but the acid plates solidified on cooling, and generally the result was satisfactory. The possibility of chemical action in the autoclave between the acid or alkali and the medium was reduced, and in the case of hydrochloric acid there was less chance of loss through volatilization.

The culture medium used for a set of dishes of any one series was taken from the same stock flask to obviate the introduction of differences present in the stock media.

Similarly, the inoculations of each complete series were all made from one stock culture, by transferring 3 mm. cubes of the medium with the mycelium attached.

The growth of the fungus in the toxicity series was measured from the inoculum to the circumference of the growing circle of mycelium. As a general rule, this circle had a well-defined margin, and was very regular in outline. In the control dishes the mycelium formed a thick white felt, closely adpressed to the medium.

The following substances were added to the media in the toxicity series as volumes of normal solution :—Hydrochloric acid (HCl), sulphuric acid (H_2SO_4), citric acid ($C_6H_8O_7$), caustic soda ($NaOH$), and caustic potash (KOH). Lime was added as slaked lime ($Ca(OH)_2$) in weighed quantities, and as concentrated lime water solution. Slaked lime was used, as quicklime on addition to the medium would have immediately become slaked lime. Slaked lime further affords a better comparison with the quicklime as applied in the field, of which a considerable proportion under Ceylon conditions would be present as slaked lime before application, and in any case would be immediately hydrated in contact with soil moisture.

The chemical formulæ for the above substances (in brackets) have been inserted here for reference, as in the construction of tables later on it was found convenient to employ these abbreviations.

Toxicity Tests.

In the following tables the volume of normal solution added to the culture is given in the first column. In the

subsequent columns the particular alkali or acid is stated, and under this is given the growth of the fungus mycelium at the various concentrations :—

SERIES 1.—Maize Meal Agar. Acid and Alkali added to medium before autoclaving. Mycelium measured after six days' growth.

Normal Solution. cc.	NaOH. cm.	HCl. cm.
0·2	4·0	Nil
0·4	2·2	Nil
0·6	1·0	Nil
0·8	0·4	Nil
1·0	Nil	Nil
1·2	Nil	Nil
1·4	Nil	Nil
2·0	Nil	Nil

Control 2·5 cm.

In the alkaline dishes the lowest concentration of alkali stimulated the fungus to greater growth than the control, while twice this concentration reduced the growth to the equivalent of the control dish. With increasing concentration, the increasing toxic effect is marked, till total inhibition occurs at a concentration of five times the lowest.

None of the acid dishes set solid on cooling, and though the liquid media may have had a deterrent effect on growth, the fact that no growth occurred indicates that acid media are unfavourable to the growth of the fungus.

It would appear from this experiment that the optimum point for the growth of the fungus is about ·02 cc. of caustic soda solution per 12 cc. of medium, or a concentration of ·07 per cent. by weight of caustic soda.

SERIES 2.—Maize Meal Agar. Acid and Alkali added to medium before autoclaving. Mycelium measured after seven days' growth.

Normal Solution. cc.	NaOH. cm.	KOH. cm.	HCl. cm.	H_2SO_4 cm.	$C_6H_8O_7$. cm.
0·1	0·8	0·7	Nil	Nil	0·9
0·2	1·9	0·5	Nil	Nil	0·2
0·4	1·1	0·4	Nil	Nil	Nil
0·6	0·9	0·3	Nil	Nil	Nil
0·8	Nil	Nil	Nil	Nil	Nil

Control 2 cm.

Alkali dishes measured again after twenty days' growth :—

Normal Solution. cc.	NaOH. cm.	KOH. cm.
0·1	4·8	4·6
0·2	4·8	4·7
0·4	2·5	2·5
0·6	3·0 (*a*)	3·0 (*a*)
0·8	0·5	0·5

Control 4·6 cm.

The alkaline dishes supplied more favourable conditions for growth than the acid dishes. In the 0·1 cc. NaOH and KOH dishes the growth was slower at the beginning than the control, but later caught up with it. In the 0·2 cc. NaOH the growth equalled the control all through. The 0·2 KOH behaved similarly to the 0·1 KOH. In both cases the toxic effect of the alkali was felt at higher concentrations.

The growth on all dishes was thin, but the control showed the thickest felt of mycelium of any in the series. In 0·4 KOH and above, and 0·2 NaOH and above, the mycelium was almost invisible to the naked eye. In the dishes marked (*a*) there was no felted mycelium, growth consisting only of scattered hyphæ.

All the acid dishes, except citric acid ($C_6H_8O_7$) 0·1 cc., were liquid. Though two mould fungi were found growing in a normal solution of citric acid, it is to be noted that 0·4 cc. inhibited all growth of *Fomes* mycelium, *i.e.*, the mould fungi could grow in a concentration thirty times that sufficient to stop all growth of *Fomes*.

In this series the toxic effect of the alkalis was noticeable at the lowest concentrations, in so far as these yielded a much thinner growth of mycelium than the control.

SERIES 3.—Bean Seed Agar. Acid and Alkali added to medium before autoclaving. Measured after six days' growth.

Normal Solution. cc.	NaOH. cm.	KOH. cm.	HCl. cm.	H_2SO_4. cm.	$C_6H_8O_7$. cm.
0·1	1·6	1·7	2·3	Nil	0·5
0·2	2·5	1·2	Nil	Nil	Nil
0·4	1·6	1·0	Nil	Nil	Nil
0·6	0·6	0·6	Nil	Nil	Nil
0·8	Nil	Nil	Nil	Nil	Nil

Control No. 1, 2·1 cm. ; No. 2, 2·0 cm.

The acid dishes each had an extra ·3 gm. of agar added, and solid media were obtained in $C_6H_8O_7$ 0·1 and 0·2, HCl 0·1, and H_2SO_4 0·1. Growth in all dishes was very thin, the thickest growth of the series being in HCl 0·1. However, as acid dishes had an addition of agar, and as the agar is alkaline in reaction, the true acidity of the acid dishes does not correspond with the values in the first column. In point of fact, the HCl 0·1 dish must have been reduced considerably in acidity, and would approximate the normal. The readings under HCl 0·1 and $C_6H_8O_7$ 0·1 cannot therefore be accepted. It is noteworthy that no growth occurred in the H_2SO_4 dishes, nor in the higher HCl and $C_6H_8O_7$ dishes, in spite of their reduced acidity.

With regard to the alkaline dishes, an optimum alkaline point apparently exists about concentration 0·2 cc. caustic soda as in Series 1, while in the caustic potash dishes the toxicity steadily increases with higher proportions of the alkali.

Series 4.—Bean Seed Agar. Alkali added before autoclaving: acid added afterwards. Requisite volumes of distilled water in both cases added beforehand. Measured after seven days' growth.

Normal Solution.	NaOH.	KOH.	HCl.	H_2SO_4.	$C_6H_8O_7$.
cc.	cm.	cm.	cm.	cm.	cm.
0·1	2·0	0·4	0·9	0·1	1·9
0·2	2·2	1·1	Nil	Nil	0·7
0·4	1·8	0·9	Nil	Nil	Nil
0·6	0·8	0·6	Nil	Nil	0·1
0·8	0·7	0·5	Nil	Nil	Nil

Control No. 1, 3·2 cm.; No. 2, 2·6 cm.

All media were solid. The thickest growth was obtained on Control No. 1, while No. 2 was not as thick as $C_6H_8O_7$ 0·1 and 0·2, and HCl 0·1. In the case of $C_6H_8O_7$ 0·4 and H_2SO_4 0·1 the growth was aerial on the inoculum, and not in contact with the acid medium. The growth on the alkali dishes was thin, being thinner than the first two citric acid and the first hydrochloric acid dishes.

At all concentrations, except the lowest, the alkaline media are markedly less toxic to the fungus than the acid media.

SERIES 5.—Requisite quantities of water added and medium autoclaved; Acid and Alkali added afterwards. Mycelium measured after the number of days of growth as indicated.

French Bean Agar.

Normal Solution. cc.	NaOH. 3 Days. cm.	NaOH. 6 Days. cm.	NaOH. 10 Days. cm.	NaOH. 14 Days. cm.	HCl. 3 Days. cm.	HCl. 6 Days. cm.	HCl. 10 Days. cm.	HCl. 14 Days. cm.
0·2	0·5	0·7	1·1	2·0	0·7	1·6	2·1	2·5
0·4	0·5	0·7	0·9	1·1	Nil	Nil	Nil	Nil
0·6	0·2	0·5	0·6	0·7	Nil	Nil	Nil	Nil
0·8	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
1·0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Control	1·1	2·0	2·5	3·0				

Maize Meal Agar.

Normal Solution. cc.	NaOH. 3 Days. cm.	NaOH. 6 Days. cm.	NaOH. 10 Days. cm.	NaOH. 14 Days. cm.	HCl. 3 Days. cm.	HCl. 6 Days. cm.	HCl. 10 Days. cm.	HCl. 14 Days. cm.
0·2	Nil	Nil	Nil	0·5	Nil	Nil	Nil	Nil
0·4	Nil	Nil	Nil	0·2	Nil	Nil	Nil	Nil
0·6	Nil	Nil	Nil	0·5	Nil	Nil	Nil	Nil
0·8	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
1·0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Control	0·2	1·4	1·6	3·0				

Richard's Solution (3)* with 20 gm. agar per litre added.

Normal Solution. cc.	NaOH. 3 Days. cm.	NaOH. 6 Days. cm.	NaOH. 10 Days. cm.	NaOH. 14 Days. cm.	HCl. 3 Days. cm.	HCl. 6 Days. cm.	HCl. 10 Days. cm.	HCl. 14 Days. cm.
0·2	Nil	Nil	0·7	0·8	Nil	Nil	Nil	Nil
0·4	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
0·6	N l	Nil	Nil	Nil	Nil	Nil	Nil	Nil
0·8	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
1·0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Control	Nil	0·4	1·0	1·2				

* Zeller, Schmitz, and Duggar. Growth of Wood-destroying Fungi in Liquid media. Ann. Miss. Bot. Gdn., VI., p. 148.

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This series of three parallel sets on different media was set up to determine the growth on different media under the same toxic concentrations.

French bean agar, which gives the best growth in neutral media, induced growth at concentrations which were toxic in the case of Richard's medium, and at which, in the maize meal agar, growth occurred only at a late period. In this respect the behaviour of the 0·2 cc. HCl in French bean agar is noteworthy, as this is the only record of growth on any medium at this concentration of hydrochloric acid.

SERIES 6.—Lima Bean Agar. Requisite quantities of distilled water added before autoclaving. Acid and Alkali added afterwards. Measured after seven days' growth.

Normal Solution. cc.	NaOH. cm.	KOH. cm.	HCl. cm.	H_2SO_4. cm.	$C_6H_8O_7$. cm.
0·1	3·7	X	2·2	0·2	1·6
0·2	1·6	0·6	Nil	Nil	2·3
0·4	0·6	0·1	Nil	Nil	Nil
0·6	0·1	0·1	Nil	Nil	Nil
0·8	Nil	Nil	Nil	Nil	Nil

Control No. 1, 3·0 cm.; No. 2, 2·8 cm.

The dish marked with a X was contaminated and was discarded. The acid dishes, except H_2SO_4 0·1 where the growth was only on the inoculum, produced a thicker and more closely felted growth than any of the other dishes, including the controls.

In this series the fact that the acids and alkalis were added after autoclaving reduced to a minimum the possibility of chemical reaction between these substances and the nutrient medium. In the case of the caustic alkalis, reaction with the proteins of the nutrient medium resulting in the formation of sodium sulphide would probably not occur, and consequently all the caustic alkali added as normal solution would be present in the medium as caustic alkali. The concentrations represented by the values in the first column would, therefore, be greater than in preceding series, where the medium was autoclaved with the alkali present. This may account for the reduction of the optimum point of growth in NaOH media from 0·2 to 0·1. The acid series in all concentrations above the lowest showed total inhibition of growth. The case of citric acid is exceptional, as the acid itself is a possible nutrient substance for the fungus.

SERIES 7.—Bean Agar with slaked lime. Lime added before autoclaving. Measured after days' growth as indicated.

$Ca(OH)_2$ gm.	Growth. 2 Days. cm.	5 Days. cm.	9 Days. cm.	13 Days. cm.	20 Days. cm.
0·05	0·2	0·3	0·8	1·2	2·2
0·10	Nil	0·05	0·1	1·0	1·7
0·15	Nil	Nil	Nil	Nil	Nil
0·20	Nil	Nil	Nil	Nil	Nil
0·25	Nil	Nil	Nil	Nil	Nil
Control	0·7	2·0	3·0	3·5	4·7

A saturated solution of lime water contains 0·17 gm. $Ca(OH)_2$ in 100 cc.; to give a saturated solution in 12 cc. of culture medium 0·02 gm. of $Ca(OH)_2$ would be required. With 0·05 gm. of $Ca(OH)_2$ the medium is, therefore, saturated, and about half of the $Ca(OH)_2$ is in suspension. Similarly, in the higher values of $Ca(OH)_2$, part goes to form a saturated solution, and the remainder is held in suspension in the medium or settles to the bottom of the dish as a sediment. It is evident from the table that sufficient lime comes in contact with the fungus to inhibit all growth at the higher values, and that with increasing quantities of lime there is an increasing toxic effect on the fungus.

SERIES 8.—Bean Agar with slaked lime and carbonate of lime. All substances added before autoclaving.

Quantity. gm.	$Ca(OH)_2$. 7 Days. cm.	10 Days. cm.	29 Days. cm.	$CaCO_3$. 7 Days. cm.	10 Days. cm.	29 Days. cm.
0·01	2·0	3·0	4·2	1·8	3·4	4·2
0·02	1·2	1·7	3·1	2·1	3·3	4·7
0·05	0·2	0·3	0·7	1·9	3·0	4·0
0·10	Nil	Nil	1·0	1·4	1·4	4·5
0·25	Nil	Nil	Nil	1·8	3·0	4·8
0·50	Nil	Nil	Nil	2·0	4·7	4·8
Control	1·2	3·5	4·3			

On $CaCO_3$ 0·5 the growth was thickest, the control coming next. Good growth was obtained on all the $CaCO_3$ dishes, the 0·50 dish of which was practically full of mycelium after ten days' growth. It must, however, be pointed out that the carbonate of lime in the 0·25 and 0·50 dishes had for the most part settled on the bottom of the dish as a sediment, and was therefore inactive as concerned the growth of the fungus. The same remark applies to the 0·25 slaked lime dish. In the remaining carbonate and slaked lime dishes

a fairly good suspension was obtained. It will be noted that the fungus is indifferent to carbonate of lime, but is strongly inhibited in growth with increasing proportions of slaked lime.

From this and the preceding series, it is apparent that growth is totally inhibited at about 0·15 gm. $Ca(OH)_2$ in 12 cc., or 11·25 gm. weight of medium, *i.e.*, at a concentration of 1·3 per cent. by weight of $Ca(OH)_2$.

SERIES 9.—Bean Agar with slaked lime and carbonate of lime. All substances added before autoclaving. Measured after six days' growth.

Quantity. gm.	$Ca(OH)_2$. cm.	$Ca(CO)_3$. cm.
0·01	1·7	2·8
0·02	0·7	2·1
0·05	Nil	2·7
0·10	Nil	2·4
0·25	Nil	2·6
1·00	Blank	1·5
5·00	Blank	2·5

In this series the fungus again is indifferent to the presence of carbonate of lime in the medium. The toxic effect in the slaked lime dishes is apparent at a lower concentration than in the preceding series, and at double the saturation value of the medium, *i.e.*, at 0·05 gm. $Ca(OH)_2$ no growth has occurred.

SERIES 10.—Bean Agar. All substances added after autoclaving. Measured after seven days' growth.

Lime Water. cc.	Wt. of $Ca(OH)_2$ gm.	Water. cc.	Growth. cm.	
1	·0017	1	1·5	Control : 3·0 cm. ; 2·8 cm.
2	·0034	Nil	2·7	
3	·0051	2	1·8	For inter-comparison.
4	·0068	1	0·5	
5	·0085	Nil	0·5	

SERIES 11.—Maize Meal Agar with lime water. All substances added before autoclaving. Measured after ten days' growth.

Lime Water. cc.	Wt. of $Ca(OH)_2$. gm.	Water. cc.	Growth. cm.
1	·0017	4	2·5
2	·0034	3	1·4
3	·0051	2	3·4
4	·0068	1	2·1
5	·0085	Nil	0·7
Control Nil	Nil	5	3·0

From series 10 and 11 it would appear that lime water, *i.e.*, slaked lime in solution, exhibits no toxic effect on the fungus below a concentration of ·0085 gm. in 15 cc. of medium, *i.e.*, a concentration of ·06 per cent. by weight of slaked lime.

SERIES 12.—Bean Agar and Maize Meal Agar; 10 cc. medium in each dish. Slaked lime added before autoclaving. Measured after ten days' growth.

Quantity. gm.	Bean Agar. cm.	Maize Agar. cm.
0·01	0·3	0·9
0·02	2·1	1·7
0·05	2·1	0·7
0·10	Nil	Nil
0·50	Nil	Nil
Control No. 1	1·7	1·4
No. 2	0·6	1·9

Growth here was more vigorous on bean agar than on maize agar, and, compared with the control, was stronger on the 0·02 and 0·05 concentrations than in Series 7 and 8. The point of total inhibition remains about the same however.

SERIES 13.—Bean Agar. Autoclaved and poured. Thick paste made up with water and carbonate of lime, sterilized, and poured in the shape of a ring round the inoculum on the inoculated dishes. Similar rings poured with paste of slaked lime and water.

$CaCO_3$ Rings :—

(1) Hyphæ crossed ring in eight days and growing beyond.
(2) Hyphæ crossed ring in eight days and growing beyond.
(3) Hyphæ in contact with ring in eight days, crossed in twelve days.

$Ca(OH)_2$ Rings :—

(1) Hyphæ not crossed in eight days ; growth 0·2 cm.
(2) Hyphæ not crossed in eight days ; growth 0·2 cm.
(3) Hyphæ not crossed in eight days ; growth 0·1 cm.

After twelve days' growth, hyphæ crossed $Ca(OH)_2$ ring No. 2 and continued growth beyond. It appears that the fungus hyphæ can continue growth even when in actual contact with slaked lime.

SERIES 14.—Soil Cultures. Fresh damp loamy soil sifted, and 12 gm. portions put in Petri dishes and autoclaved for 40 minutes at 115° C. on two successive days. Weighed quantities of $Ca(OH)_2$ and $CaCO_3$ were then added, and the whole well mixed by stirring. Inoculations made from bean agar culture.

Quantity. gm.	$Ca(OH)_2$. 4 Days. cm.	$Ca(OH)_2$. 6 Days. cm.	$CaCO_3$. 4 Days. cm.	$CaCO_3$. 6 Days. cm.
0·03	1·1	1·4	1·1	1·7
0·10	0·6	0·8	1·1	1·6
0·50	Nil	Nil	1·3	1·8
1·0	Nil	Nil	1·3	1·5
5·0	Nil	Nil	1·1	1·8
Control	1·3	1·7		

The toxic concentration of slaked lime is apparently higher in soil than in culture media. Adsorption due to soil colloids and to the presence of insoluble solid particles in the soil-lime mixture would tend to reduce the toxicity of the slaked lime. In the nutrient media, agar being a colloid would also cause a reduction of toxicity. The reduction in toxicity in soil would appear to be greater, as the toxic effect of 0·10 gm. is less in soil than in culture media.

DISCUSSION.

The degree of acidity or alkalinity of the medium may be expressed by Fuller's scale, where + 1 signifies 1 cc. of normal acid per litre, and — 1 signifies 1 cc. of normal alkali per litre, and so on with 2, 3, &c. The equivalents on Fuller's scale of the quantities used in the preceding experiments are as follows :—

Normal Solution. cc.		Fuller's Scale.
0·1	=	8
0·2	=	16
0·4	=	33
0·6	=	50
0·8	=	66

The fungus thus is capable of exhibiting growth on media up to — 66 Fuller's scale, but grows with difficulty at + 8 Fuller's scale, and is totally inhibited at + 16. The case of Citric acid may be neglected, as the acid itself may act as a source of nutrition for the fungus. At — 8 Fuller's scale growth generally was equal to that on the neutral control dishes. The fungus, therefore, is capable of growth under a greater range of alkaline conditions than of acid conditions.

With slaked lime total inhibition of growth occurred at a concentration of about 0·15 gm. per 11·25 gm. of medium, or 1·33 per cent. of slaked lime. Taking the top 8 inches of soil as weighing 2,000,000 lb. per acre and the area occupied by one tree as 480 square feet, then the weight of the top 8 inches of soil round one tree is 22,000 lb. To obtain a concentration of 1·3 per cent. of slaked lime in this quantity of soil 292 lb. would be required for each tree, or roughly five times the quantity usually applied.

In Europe good burnt lime contains from 80 per cent. to 90 per cent. of caustic lime, and about 2 per cent. of carbonate of lime. In Ceylon burnt coral lime contains only 25 per cent. of caustic lime and 75 per cent. of carbonate of lime.* The fungicidal effect of burnt lime on *Fomes lignosus* mycelium depends on the caustic action of caustic lime in direct contact with the mycelium, and on the degree of alkalinity subsequently produced in the soil. Both these factors vary directly as the proportion of caustic lime in burnt lime; the lower proportion in Ceylon burnt coral thus results in a correspondingly reduced fungicidal value. Carbonate of lime has no deterrent effect on the growth of the fungus.

Burnt lime when applied to soil is converted to carbonate of lime. The steps of this process are, first, hydration of the caustic lime to slaked lime in the presence of soil moisture; and, second, carbonation of the slaked lime to carbonate in the presence of carbon dioxide in the soil. The end product, carbonate of lime, in the above changes is inactive with regard to the growth of the fungus. The duration of the fungicidal effect of an application of burnt lime, therefore, depends on the rate at which the active substances are converted to inactive, *i.e.*, the rate of change from caustic lime to carbonate of lime.

MacIntire † has shown that caustic lime is rapidly hydrated to slaked lime in a moist atmosphere, small quantities being completely hydrated in one night. Under Ceylon conditions, it follows that in burnt lime the caustic lime is usually to a large extent already hydrated to slaked lime, before the lime arrives on the estate, and to a greater extent by the time the lime is applied in the field. The caustic property of the burnt lime is accordingly reduced, and it may reasonably be assumed that in many cases no caustic effect, due to contact of caustic lime with the mycelium, is produced.

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* Government Agric. Chem. Ceylon. Communicated.

† MacIntire, W. H. The carbonation of burnt lime in soils. Soil Science, VII. p., 325. 1919.

There remains the most important phase of the fungicidal effect of applications of burnt lime to the soil, viz., the slaked lime phase. Slaked lime is slightly soluble in water, and thus soil water percolating through the soil may carry down slaked lime in solution to the deeper layers. It is evident, however, from the culture experiments with slaked lime and with lime water, that even saturated solutions of slaked lime do not produce total inhibition of growth, and less concentrated solutions have reduced effects. Solutions of slaked lime percolating through the soil undergo rapid diminution in concentration due to the formation of carbonate, to chemical reaction with the soil, and to adsorption by the soil colloids. Any slaked lime solution thus reaching the fungus mycelium in the deeper layers of the soil would have little, if any, inhibitive effect on the fungus.

The burnt lime is forked into the top 6 or 8 inches of soil, where it undergoes conversion to carbonate. MacIntire has shown that the maximum carbonation in the top 6 inches of soil occurs within five days after the application in the case of applications at the rate of 2 tons of burnt lime (80 per cent. caustic lime) per acre. With heavier applications the maximum carbonation takes longer. The process of carbonation, or conversion of slaked lime to carbonate, however, occurs in such a way that the fungicidal value of the burnt lime rapidly falls to zero before complete carbonation has occurred. MacIntire demonstrates that a film of carbonate of lime is formed round the particles or lumps of burnt lime, and this acts as a protective crust which retards the conversion of the interior to carbonate. To all intents and purposes, as regards the fungicidal value of burnt lime on *Fomes lignosus*, as soon as this crust is formed the lime is equivalent to carbonate and its value accordingly is nil.

Fomes lignosus mycelium is very susceptible to acid conditions, and is somewhat resistant to alkaline conditions. The direct fungicidal value of an application of burnt lime depends on the proportion of caustic lime or slaked lime in it, and in any case is ephemeral with regard to *Fomes lignosus*. The after effects of applications of burnt lime are to render the soil more alkaline and thus more favourable to the fungus. Under these circumstances, the application of burnt lime to soil to inhibit growth of *Fomes lignosus* mycelium is not to be recommended.

Until further experimental work is carried out with soil fungicides for this fungus, the other methods of treatment should be more rigorously applied. The root system of diseased trees should be removed from the soil as completely

as possible, the isolation trench should be kept open and clear of weeds and decaying vegetable matter, and the soil within the trench should be forked over at frequent intervals and thus exposed to sun and air.

Summary.

(1) *Fomes lignosus* mycelium is capable of growing on alkaline media up to — 66 on Fuller's scale.

(2) The mycelium is very sensitive to acid media, growing with difficulty at + 8 Fuller's scale, and undergoing total inhibition of growth at + 16.

(3) It is affected by slaked lime, but total inhibition does not occur until a concentration of 1·3 per cent. by weight of slaked lime is reached.

(4) Carbonate of lime has no effect on the fungus.

(5) In view of recent soil studies, which demonstrate that quicklime if applied to the soil is rapidly converted to carbonate, and in view of the foregoing results, it is considered that soil applications of burnt lime are of little fungicidal value for *Fomes lignosus*.

(6) Further experiments with soil fungicides for this fungus are necessary. Meanwhile the other operations for the control of the disease should be more rigorously applied.

G. BRYCE.

August, 1922.

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